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Case Report

Successful intrauterine treatment of a patient with cobalamin C defect

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ABSTRACT

Cobalamin C (cblC) defect is an inherited autosomal recessive disorder that affects cobalamin metabolism. Patients are treated with hydroxycobalamin to ameliorate the clinical features of early-onset disease and prevent clinical symptoms in late-onset disease. Here we describe a patient in whom prenatal maternal treatment with 30 mg/week hydroxycobalamin and 5 mg/day folic acid from week 15 of pregnancy prevented disease manifestation in a girl who is now 11 years old with normal IQ and only mild ophthalmic findings. The affected older sister received postnatal treatment only and is severely intellectually disabled with severe ophthalmic symptoms. This case highlights the potential of early, high-dose intrauterine treatment in a fetus affected by the cblC defect.

1. Introduction

Cobalamin C (cblC) defect is the most common disorder that affects intracellular cobalamin metabolism. The conversion of dietary cobalamin into its two metabolically active forms, adenosylcobalamin and methylcobalamin is impaired, resulting in elevated homocysteine (Hcy) and methylmalonic acid concentrations in body fluids [1]. A number of mutations in the *MMACHC* gene have been characterised resulting in symptomatic cblC defect, which is inherited in an autosomal recessive manner [2]. Clinical features comprise failure to thrive, mental retardation, visual impairment, hydrocephalus and congenital cardiac anomalies. Early treatment with hydroxycobalamin (OHCbl), betaine and folate has a positive impact on survival and severe organ damage. However, visual impairment and neurocognitive disability can rarely be prevented in early-onset disease [1]. Here we report a favourable outcome in the younger of two affected female siblings following prenatal treatment with 30 mg/week OHCbl from week 15 of pregnancy until delivery.

2. Patients and methods

Biochemical and enzymatic prenatal diagnosis was performed at 15 weeks of gestation during the second pregnancy of a woman who had two years previously given birth to a female child (sibling 1) with severe early-onset cblC defect. This child exhibits multiple developmental problems despite treatment with OHCbl, betaine and folic acid from week 4 of life [4]. She is now 16 years of age, unable to walk and to speak, and has severe visual impairment and behavioural problems. The parents are of Caucasian origin, no consanguinity. She has one older non-affected sibling.

Following confirmation of cblC defect in the fetus during the second pregnancy, the mother was treated from week 15 of gestation with intramuscular OHCbl (3 × 10 mg/week) and folic acid (5 mg/day) after information that this treatment was an individual treatment attempt. Postnatal treatment of sibling 2 consisted of oral betaine 200 mg/kg BW/day, folic acid 5 mg/day and intramuscular OHCbl 1 mg/day. At last follow-up at the age of 11 years, the child was treated with 2 × 10 mg intramuscular OHCbl per week, oral betaine 180 mg/kg/day and folic acid 5 mg/day (Table 1c).

Biochemical analyses of serum total Hcy (tHcy), propionylcarnitine and methylmalonic acid in urine were performed by tandem MS and HPLC/MS/MS respectively (Zentrum für Stoffwechseldiagnostik Reutlingen GmbH, Reutlingen, Germany). Enzymatic assessment and mutational analysis of the *MMACHC* gene were performed as described elsewhere [3] (Fig. 1).

Abbreviations: cblC, cobalamin C; Hcy, homocysteine; OHCbl, hydroxycobalamin; tHcy, total homocysteine; MeCbl, methylcobalamin; AdoCbl, adenosylcobalamin; CNCbl, cyanocobalamin.

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Table 1
a: Prenatal and postnatal metabolic parameters in sibling 2, treated from week 15 of gestation with maternal intramuscular OHCbl (3 × 10 mg/week) and oral folic acid (5 mg/day). b: Summary of biochemical and clinical findings in sibling 1 and 2 and c: actual treatment.

a) Biochemical prenatal (15th week of gestation) and postnatal findings	Sibling 2	Normal
Propionylcarnitine in amniotic fluid (13 weeks of gestation)	3.8 μmol/l	0.8–1.8 μmol/l (n = 5)
Methylmalonic acid in amniotic fluid	11.7 μmol/l	<0.7 μmol/l (n = 5)
Vit B12 in serum under treatment (mother) 20 weeks of gestation	>20,000 pg/ml	160–1100 pg/ml
Homocysteine in cord blood	45.1 μmol/l	<8 μmol/l
Homocysteine in serum at day 1	27.3 μmol/l	<12 μmol/l
Vit B12 in serum at day 1	>20,000 pg/ml	160–1100 pg/ml
Homocysteine in serum day 7	41.7	<12 μmol/l
Propionylcarnitine in dried blood at day 1	5	<6.8
Methylmalonic acid excretion in urine at day 7	66 mmol/mol creatinine	0–10 mmol/mol creatinine

b) Biochemical findings postnatal	Sibling 1	Sibling 2	Normal
Methylmalonic acid in urine at 20 days of age	1940 mmol (mol creatinine)	60 mmol/mol creatinine	0–10 mmol/mol creatinine
Propionylcarnitine at 20 days/at day 1	32	5	<6.8
Homocysteine in serum at 20 days of age	282 μmol/l	41	<8
Actual[a] methylmalonic acid in plasma	9.1 μmol/l	2.7 μmol/l	<0.26 μmol/l
Actual[a] homocysteine serum	Not available	72 μmol/l	<8
Actual[a] methionine in serum	37 μmol/l	23 μmol/l	15–45 μmol/l
Actual[a] methylmalonic acid in urine	Not available	17.6 mmol/mol creatinine	0–10 mmol/mol creatinine

Clinical findings	Sibling 1	Sibling 2
Weight at birth	2570 (1st perc.)	3100 (10th perc.)
Head circumference at birth	33(1st perc.)	35 (25th perc.)
General findings at infancy/at birth	Hypotonia, feeding problems, heart murmur at day 20, myoclonic seizures, horizontal nystagmus, respiratory distress	Normal clinical findings
MRI of brain at day 29/day 7	Diffuse supratentoric cortical atrophy without signs of hypoxic-ischaemic lesions	Slightly delayed white matter maturation and asymmetric increased signal intensities of the white layer
Actual[a] development test, IQ	Denver test corresponds to 8–11 months of age	WISC-IV total IQ 103
Actual[a] somatic status	32 kg (z-score −5), height ca. 140 cm (<3rd perc.), HC 48 cm (<3rd perc)	Height 150 cm (47th perc.), weight 34 kg (21st perc.), HC 52.5 (9th perc.)
Ophthalmic findings at 3 months of age sibling 1	Bil loss of photoreceptors in the central region of the macula and partial optic atrophy [4]	Not investigated
Actual[a] ophthalmic findings	Bil nystagmus, bil disc pallor, fundi? (no cooperation)	Acuity (distance) 20/30, no nystagmus, minor marginal disc pallor, normal fundi
c) Actual[a] treatment		
Hydroxycobalamin	2 × 10 mg i.m./week	2 × 10 mg i.m./week
Folic acid	5 mg/day	5 mg/day
Betaine	180 mg/kg BW/day	180 mg/kg BW/day
Pipamperon	3 × 20 mg p.o./day	
Atomoxetine	1 × 18 mg p.o./day	

[a] Actual: sibling 1: 16 years, sibling 2: 11 years of age.

3. Results

Biochemical and clinical findings are summarised in Table 1a–c. No complications during pregnancy and delivery of the female sibling 2 following intrauterine treatment were reported and the infant appeared to be healthy and without congenital anomalies. MRI of the brain after birth showed slightly delayed white matter maturation and asymmetric increased signal intensities of the white layer (Table 1b). In contrast, sibling 1 showed diffuse supratentoric cortical atrophy without signs of hypoxic-ischaemic lesions and eye findings revealed bilateral loss of photoreceptors in the central region of the macula and partial optic atrophy at 29 days of age (Table 1b). Data of the very different psychomotor development in both siblings are shown in Table 1b. Sibling 2 attends secondary school with excellent success and has a total IQ of 103 (WISC-IV). The affected older sibling shows in the Denver test a corresponding development of an 8–11 months infant even treated from 4 weeks of age with OHCbl, betaine and folic acid as described [4]. For details we refer to reference [4]. She is unable to walk, lacks speech development and shows an aggressive behaviour which is partly controlled by pipamperon and atomoxetine (Table 1c).

Biochemical results from amniotic fluid at gestational week 15 as well as postnatal serum and urine for sibling 2 are summarised in Table 1a. Values for methylmalonic acid and homocysteine were pre- and post-natally elevated. However, methylmalonic acid excretion over the years remained below 60 mmol/mol creatinine (normal, <20 mmol/mol) and serum tHcy between 40 and 60 μmol/l (reference, <12 μmol/l).

Both affected children share the same genotype and similar residual enzymatic activity (Table 2). Total uptake of [^{57}Co]cyanocobalamin was strongly reduced compared to values of 32 control individuals (Table 2). The percentage of methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) was reduced in comparison to the mean control value, the percentage of cyanocobalamin (CNCbl) was increased and the percentage of OHCbl was in range of the control values for both children (Table 2).

Monitoring of serum tHcy over time shows values <100 μmol/l in both siblings (in sibling 1 from 3 months of age, Fig. 2). However, variations in tHcy were generally higher in the girl without prenatal OHCbl treatment.

Methylmalonic acid in urine was only slightly elevated in both siblings (Table 1). Decrease of methylmalonic excretion in urine and methionine concentration in serum of sibling 1 is shown in Fig. 3 indicating rapid response to the treatment in sibling 1.

4. Discussion

The high rate of congenital anomalies, including heart defects and CNS involvement, observed in early-onset cblC defect patients suggests that enzymatic dysfunction during embryonal and/or fetal stages of development contribute to the clinical phenotype [1] and that postnatal treatment alone may not be sufficient to support normal psychomotor development and prevent ocular damage [5, 6]. The outcome in our prenatally treated patient reported here with cblC defect was much more favourable than in a case previously described [7], in which prenatal treatment was initiated at a lower dose and at a later gestational stage (2 mg/week OHCbl from week 25 compared to 30 mg/week OHCbl from week 15 in our patient). In the case described by Huemer et al. [7] retinopathy and cognitive impairment could not be prevented and tHcy was highly elevated (160.9 μmol/l) in a neonate despite prenatal treatment. Methylmalonic acid excretion was also significantly elevated (393 mmol/mol creatinine). In our patient, tHcy was 27.3 μmol/l and methylmalonic acid excretion was 66 mmol/mol creatinine after delivery, suggesting a better therapeutic effect associated with the higher dosage of OHCbl on the fetus (Table 1a).

Both the low level of enzymatic activity and the *MMACHC* locus genotype (c.457C>T, p.Arg153*/c.271dupA, p.Arg91Lysfs*14) suggest a

Fig. 1. A) Normally developed sibling 2 at the age of seven years (prenatal and postnatal treatment); B) severely intellectually disabled sibling 1 at the age of 12 years (postnatal treatment only).

Table 2
Comparison of intracellular metabolic processing of CNCbl (fibroblasts) in siblings with identical genotype compared to control individuals (n = 32). AdoCbl, adenosylcobalamin; CNCbl, cyanocobalamin; MeCbl, methylcobalamin; OHCbl, hydroxycobalamin.

	Patient ID 160	Patient ID 959	Control value mean (range)
[^{57}Co]cyanocobalamin uptake (pg/mg protein)			
Total uptake	8.8	10	71 (19–142)
Cobalamin coenzyme synthesis from [^{57}Co]cyanocobalamin (distribution of cobalamins: % of total cobalamins)			
MeCbl	3.3	1.0	60 (48–78)
AdoCbl	3.8	3.5	14 (6.9–30)
CNCbl	82	77	8.7 (3.3–18)
OHCbl	10	18	16 (7.3–25)

severe congenital defect in the siblings reported here. The same genotype has been described in several patients with early-onset cblC defect [2, 8, 9] and no patients with this genotype and late-onset disorder have been described. In addition, the nonsense mutation (c.457C>T, p.Arg153*) has been described in combination with c.331C>T (p.Arg111*) in a further early-onset patient [2] and in combination with a missense mutation (c.365A>G, p.His122Arg) in patients with late-onset disease [10].

The contribution of additional factors beyond prenatal treatment that may have influenced the contrasting outcomes in the two siblings described here cannot be excluded. A large variation in clinical symptoms between siblings with late-onset disease is well known [11–13]. Augoustides-Savvopoulou et al. describe a child with early-onset symptoms of cblC defect with severe neonatal seizures, developmental delay and spastic paraparesis who died at the age of 13 years, while the child's

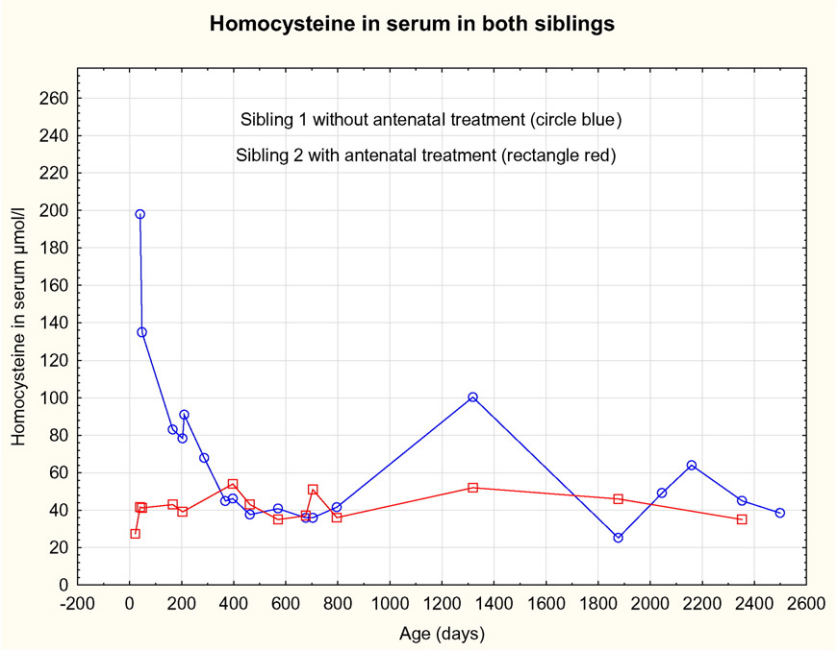

Fig. 2. Serum homocysteine concentration in two siblings with cblC defect from day 1 in the antenatally-treated patient (sibling 2) and in her older sister (sibling 1) who was detected by selective screening of organic acids in urine on day 29 [Table 2 and citation [4]].

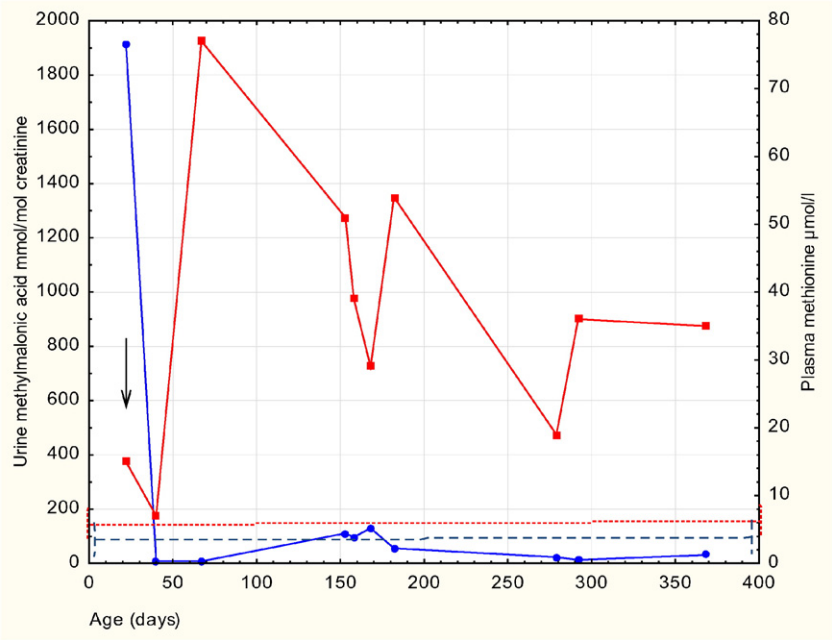

Fig. 3. Methionine in serum (red) and methylmalonic acid excretion in urine (blue) before and after initiation of therapy in sibling 1. Arrow shows start of treatment. Dashed line (red points) show lower normal limit of methionine in plasma and dashed line (line blue) shows upper limit of methylmalonic excretion in urine.

sibling developed apparently normally until the age of ten years, when a decline in cognitive abilities, ataxia and myoclonic jerks occurred [14]. Also our case (sibling 1) suffered from metabolic decompensation at 3 weeks of age which may have contributed to the unfavourable development. However, eye and cerebral MRI findings of the brain indicate that these early changes may have developed prenatally and are not likely to be caused by increased homocysteine levels in the first 3 weeks of life or by metabolic decompensation. The very rapid decrease of methylmalonic excretion in urine and normalization of plasma methionine indicate the otherwise excellent reaction on OHCbl treatment (Fig. 3). There were no different treatment modalities in both children, however, there is some uncertainty about a "safe" level of homocysteine in respect to an impact on the neurologic development. There are no long term prospective studies from birth showing the influence of elevated homocysteine on the psychomotoric/ocular development [6].

In conclusion, this very limited experience suggests that early prenatal treatment may prevent disease manifestations in patients at risk of developing a cblC defect phenotype. With successful newborn screening the possibility of improving outcome in patients with cblC defect has already been established [5, 15, 16], however prenatal diagnosis and treatment together with lifelong postnatal treatment may represent an opportunity to prevent the manifestation of clinical symptoms altogether. Our results suggest that intrauterine treatment should be initiated as early as possible in an affected fetus and continued throughout gestation. The high dosage used here resulted in a high Vit B12 concentration in the mother's as well as in the fetal blood circulation as documented at day 1 of life. The importance of high OHCbl dosages to reduce homocysteine and methylmalonic acid have been demonstrated in a 13 year old patient [17]. Further experiences in similar cases during pregnancy should be collected in the future and Vit B12 and postnatal homocysteine levels should be monitored closely in affected patients to better understand the impact of OHCbl dosage on homocysteine levels in serum [18].

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